Retroviral Infection Kit

Cell Transfection/transduction

CATALOGUE NO.: CTK002

DESCRIPTION: The MSCV (Murine Stem Cell Virus) Retroviral Expression System contains vectors that are optimized for introducing and expressing target genes in proliferating murine or human cell lines. This highly efficient system is ideal for analyzing gene expression and function in development, embryogenesis, and carcinogenesis in both cell culture and transgenic assays.

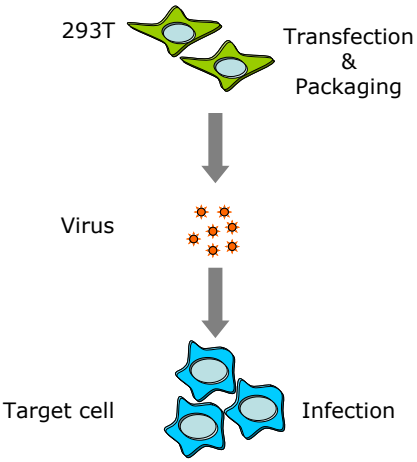
Designed for tough-to-transfect cells, the MSCV system contains two vectors: pMac-vGFP and helper that expresses protein elements required for producing high-titer virus. These vectors contain a specially designed long terminal repeat (LTR) from the murine stem cell virus that allows you to transduce most hard-to-transfect cell lines. This system drives high-level, constitutive expression of the target gene in most mammalian cell lines.

KIT COMPONENTS:

Components	*Name*	*Size*
A	HBS	100mL
B	$CaCl_2$	10mL
C	Helper	20ug in 20uL
D	pMac-vGFP	20ug in 20uL
E	Polybrene	1mL

STORAGE: Stable for >1year at 4-8°C.

PACKING SIZE: 1 kit (100 standard transfection/transduction in 10-cm plate).



Procedure illustration

EXPERIMENTAL PROCEDURE:

Day 1: Preparation of packaging cell by splitting 293T cells in 1:6-1:8 at 50% confluence

Day 2: Transfection of packaging cells

1. Preparation of DNA/CaP complex (for a 10-cm cell culture dish with 10 ml medium) (see CTK001 for details).

   In a 1.5 ml eppendorf tube, mix:

   10ug retroviral vector (expressing the gene of interest)

   20ug helper

   1 ml HBS (1x)

   67 ul CaCl2 (2M)
2. Vertex briefly (about 3 seconds).
3. Leave it in the cell culture hood for 15 minutes.

4. Pipette 3-5 times and transfer the mix to the cell culture (apply the mixture evenly to the cell).
5. Change medium after 5 hours (no more than 12 hours).
6. Prepare the cells to be infected at 30% confluence (no more than 50%).

Day 3: Harvest virus and infect cells

7. Collect cell culture medium after 36 hours.
8. Filter through 0.45 um filter.
9. Mix ½ virus + ½ fresh medium + 8 g/ml Polybrene.
10. Apply the medium containing virus particles to the cells to be infected.

Day 4: After >24 hours, check the infection efficiency by examining cells under fluorescent microscope for GFP signal.
Antiboitic selection or FACs sorting might be needed if the efficiency is lower than 80%.

M&C Gene Technology • Phone: (010)8205-7786 • (010)8693-7385 • Fax: (010)8205-9875
E-mail: order@macgene.com • Tech Support: support@macgene.com • URL: http://www.macgene.com